(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 711 158 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
03.12.2003 Bulletin 2003/49

(21) Application number: 94924519.5

(22) Date of filing: 29.07.1994

(51) Int Cl.7: A61K 31/337, A61K 33/00, A61P 9/10

(86) International application number:
PCT/US94/08578

(87) International publication number:
WO 95/003795 (09.02.1995 Gazette 1995/07)

(54) METHOD OF TREATING ATHEROSCLEROSIS OR RESTENOSIS USING MICROTUBULE STABILIZING AGENT

VERFAHREN ZUR BEHANDLUNG ATHEROSKLEROSE ODER RESTENOSE MIT HILFE EINES MIKROTUBULUSSTABILISATORS

PROCEDE DE TRAITEMENT DE L'ATHEROSCLEROSE OU DE LA RESTENOSE AU MOYEN D'UN AGENT DE STABILISATION DES MICROTUBULES

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: 29.07.1993 US 99067

(43) Date of publication of application:
15.05.1996 Bulletin 1996/20

(60) Divisional application:
00128626.9 / 1 118 325

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Description

[0001] The present invention relates to the use of paclitaxel, paclitaxel derivatives and deuterium oxide for the manufacture of a pharmaceutical composition for treating patients at risk of developing atherosclerosis.

[0002] More particularly, the invention relates to the use as described above for treating these patients with a low dose paclitaxel solution to prevent or reduce the development of atherosclerosis.

[0003] Vascular disease is the leading cause of death and disability in the developed world, particularly afflicting the elderly. In the United States alone, despite recent encouraging declines, cardiovascular disease is still responsible for almost one million fatalities each year and more than one half of all deaths; almost 5 million persons afflicted with cardiovascular disease are hospitalized each year. The cost of this disease in terms of human suffering and of material resources is almost incalculable.

[0004] Atherosclerosis is the most common form of vascular disease and leads to insufficient blood supply to critical body organs, resulting in heart attack, stroke, and kidney failure. Additionally, atherosclerosis causes major complications in those suffering from hypertension and diabetes, as well as tobacco smokers. Atherosclerosis is a form of chronic vascular injury in which some of the normal vascular smooth muscle cells ("VSMC") in the artery wall, which ordinarily control vascular tone regulating blood flow, change their nature and develop "cancer-like" behavior. These VSMC become abnormally proliferative, secreting substances (growth factors, tissue-degradation enzymes and other proteins) which enable them to invade and spread into the inner vessel lining, blocking blood flow and making that vessel abnormally susceptible to being completely blocked by local blood clotting, resulting in the death of the tissue served by that artery.

[0005] As a result, a need exists for a successful chemotherapeutic therapy to reduce or prevent artery-blockage. The most effective way to prevent this disease is at the cellular level, as opposed to repeated revascularization surgery which can carry a significant risk of complications or death, consumes time and money, and is inconvenient to the patient.

[0006] Microtubules, cellular organelles present in all eukaryotic cells, are required for healthy, normal cellular activities. They are an essential component of the mitotic spindle needed for cell division, and are required for maintaining cell shape and other cellular activities such as motility, anchorage, transport between cellular organelles, extracellular secretary processes (Dustin, P. (1980) Sci. Am., 243: 66-76), as well as modulating the interactions of growth factors with cell surface receptors, and intracellular signal transduction. Furthermore, microtubules play a critical regulatory role in cell replication as both the c-mos oncogene and CDC-2-kinase, which regulate entry into mitosis, bind to and phosphorylate tubulin (Verde, F. et al. (1990) Nature, 343:233-238), and both the product of the tumor suppressor gene, p53, and the T-antigen of SV-40 bind tubulin in a ternary complex (Maxwell, S.A. et al. (1991) Cell Growth Differen., 2:115-127). Microtubules are not static, but are in dynamic equilibrium with their soluble protein subunits, the α- and β-tubulin heterodimers. Assembly under physiologic conditions requires guanosine triphosphate (GTP) and certain microtubule associated and organizing proteins as cofactors; on the other hand, high calcium level and cold temperature cause depolymerization.

[0007] Interference with this normal equilibrium between the microtubule and its subunits would therefore be expected to disrupt cell division and motility, as well as other activities dependent on microtubules. This strategy has been used with significant success in the treatment of certain malignancies. Indeed, antimicrotubule agents such as colchicine and the vinca alkaloids are among the most important anticancer drugs. These antimicrotubule agents, which promote microtubule disassembly, play principal roles in the chemotherapy of most curable neoplasms, including acute lymphocytic leukemia, Hodgkin's and non-Hodgkin's Lymphomas, and germ cell tumors, as well as in the palliative treatment of many other cancers.

[0008] The newest and most promising antimicrotubule agent under research is paclitaxel. Paclitaxel is an antimicrotubule agent isolated from the stem bark of Taxus brevifolia, the western (Pacific) yew tree. Unlike other antimicrotubules such as colchicine and the vinca alkaloids which promote microtubule disassembly, paclitaxel acts by promoting the formation of unusually stable microtubules, inhibiting the normal dynamic reorganization of the microtubule network required for mitosis and cell proliferation (Schiff, P.B., et al. (1979) Nature 277: 665; Schiff, P.B., et al. (1981) Biochemisry 20: 3247). In the presence of paclitaxel, the concentration of tubulin required for polymerization is significantly lowered; microtubule assembly occurs without GTP and at low temperatures, and the microtubules formed are more stable to depolymerization by dilution, calcium, cold, and inhibitory drugs. Paclitaxel will reversibly bind to polymerized tubulin, and other tubulin-binding drugs will still bind to tubulin even in the presence of paclitaxel.

[0009] Paclitaxel has one of the broadest spectrum of antineoplastic activity, renewing serious interest in chemotherapeutic strategies directed against microtubules (Rowinsky, E.K., et al. (1990) Jrnl. of the Nat'l. Cancer Inst., 82: 1247-1259). In recent studies, paclitaxel has shown significant activity in advanced and refractory ovarian cancer (Einzig, A.I., et al. (1992) J. Clin. Oncol., 10:1748), malignant melanoma (Einzig, A.I. (1991) Invest. New Drugs, 9:59-64), as well as in cancers of the breast (Holmes, F.A., et al. (1991) JNCI, 83:1797-1805), head and neck, and lung.

[0010] Paclitaxel has been studied for its effect in combating tumor growth in several clinical trials using a variety of

administration schedules. Severe allergic reactions have been observed following administration of paclitaxel. However, it has been demonstrated that the incidence and severity of allergic reactions is affected by the dosage and rate of paclitaxel infusion (Weiss, R.B., et al. (1990) J. Clin. Oncol. 8: 1263).

[0011] Cardiac arrhythmias are associated with paclitaxel administration, and like allergic reactions, their incidence is affected by the dosage and rate of paclitaxel administration. Sinus bradycardia and Mobitz II arrhythmia will develop in approximately 40% and 5% of patients, respectively, beginning 4-6 hours after the start of paclitaxel infusion, and continuing for 4-8 hours after its completion. In most patients, the abnormal rhythm is transient, asymptomatic, hemodynamically stable, and does not require cardiac medications or electrical pacing. Additionally, it has been observed that the incidence of severe cardiac events is low in patients receiving paclitaxel alone. Thus, infusion times up to 24 hours have been used in treatment with paclitaxel to decrease the incidence of toxicity and allergic reaction to the drug.

[0012] During angioplasty, intraarterial balloon catheter inflation results in deendothelialization, disruption of the internal elastic lamina, and injury to medial smooth muscle cells. Finally medial VSMC dedifferentiation from a contractile to a secretory phenotype occurs. This involves, principally, VSMC secretion of matrix metalloproteinases degrading the surrounding basement membrane, proliferation and chemotactic migration into the intima, and secretion of a large extracellular matrix, forming the neointimal fibropoliferative lesion. Much of the VSMC phenotypic dedifferentiation after arterial injury mimics that of neoplastic cells (i.e., abnormal proliferation, growth-regulatory molecule and protease secretion, migration and basement invasion).

[0013] The art fails to suggest the use of paclitaxel in preventing or reducing atherosclerosis. Thus, the method of the present invention is to prevent or reduce the development of atherosclerosis using paclitaxel or a water soluble paclitaxel derivative. This microtubule stabilizing mechanism of atherosclerosis prevention is supported by the analogous results in experiments on cellular proliferation and migration using paclitaxel and ^2H_2O (deuterium oxide), which exert comparable microtubule effects via different underlying mechanisms.

[0014] Accordingly, an object of this invention is provide a pharmaceutical preparation for preventing or reducing atherosclerosis wherein said pharmaceutical preparation contains a low dosage of paclitaxel or water soluble paclitaxel derivative.

[0015] All references cited are herein incorporated by reference.

[0016] Also the following references are of particular interest: The inhibitory effect of DMSO on the proliferation of cultured arterial smooth muscle cells is discussed in Exp. and Molecular Pathology (1988), vol. 48, p. 48 - 58. The relationship between this phenomenon and the cytoplasmic microtubules is investigated. The pathophysiological mechanisms of restenosis, following coronary angioplasty are researched in J. of Int. Med. (1993), vol. 233, p. 215 - 226. In Atherosclerosis (1982), vol. 44, p. 385 - 390 the use of antitubulins for the treatment of atherosclerosis is investigated. In vivo-investigations in atherosclerotic rabbits are described in Circulation (1989), vol. 80/s-II, p. 66, whereas the benefit of a colchicine-treatment after an iliac angioplasty was researched. Normolipemic activities of acrylophenone derivatives, that have also antimicrotubular properties are described in Meth. and Find. Clin. Pharmacol. (1985), vol. 7, no. 4, p. 183 - 187. In U.S. Pat. No. 5,223,269 (Liepins) compositions of deuterium-containing compounds are disclosed that are utilized in the treatment of hypertension. In U.S. Pat. No. 5,157,049 (Haugwitz et al.) water soluble derivatives of paclitaxel are disclosed that are useful in the treatment of cancer.

[0017] In accordance with the objects of the present invention, a use as described above for preventing or reducing atherosclerosis is provided, which comprises treatment with a therapeutically effective amount of paclitaxel, a paclitaxel derivative or deuterium oxide. A therapeutically effective amount of agent is an amount sufficient to prevent or reduce the development of atherosclerosis.

[0018] This use provides an effective way of preventing or reducing the development of atherosclerosis in those patients susceptible to such disease. Additionally, because of the low dose of chemotherapeutic agent used, the chance of a patient developing adverse reactions is potentially reduced.

[0019] FIGURE 1 depicts the paclitaxel induced impairment of the ability of VSMC to invade filters coated with basement membrane proteins, and paclitaxel inhibition of cultured VSMC [^{3}H]-thymidine incorporation.

[0020] FIGURE 2 shows paclitaxel inhibition of smooth muscle cell neointimal accumulation after balloon catheter injury of the rat carotid artery.

[0021] FIGURE 3 depicts deuterium oxide dose-dependent inhibition of VSMC chemoinvasion, and deuterium oxide inhibition of cultured VSMC bromodeoxyuridine (BrDU) incorporation.

[0022] FIGURE 4 shows concentrations of paclitaxel caused dose-dependent microtubule bundling in VSMC's cultured on plastic.

[0023] FIGURE 5 shows deuterium oxide induced microtubule bundling in cultured VSCM's.

[0024] The practice of an embodiment in the present invention may be accomplished via several alternative drug delivery routes, such as intraperitoneal or subcutaneous injection, continuous intravenous infusion, oral ingestion or local (direct) delivery, or a combination of two or more. When formulating a solution for injection or continuous infusion, one must first prepare a paclitaxel solution. Paclitaxel is supplied through CTEP, DCT, NCI (IND#22850) as a concentrated solution, 6 mg/ml, in 5 ml vials (30 mg/vial) in a polyoxyethylated castor oil (Cremophor EL®) 50% and dehydrated

alcohol, USP (50%) vehicle. The intact vials should be stored under refrigeration and diluted prior to use. When diluted in either 5% Dextrose Injection or 0.9% Sodium Chloride, paclitaxel concentrations of 0.3-1.2 mg/ml are physically and chemically stable for at least 12 hours at room. temperature. (NCI Investigation Drugs; Pharmaceutical Data (1990)). It has also been demonstrated that paclitaxel concentrations of 0.6 mg/ml diluted in either D5W or NS and 1.2 mg/ml diluted in NS prepared in polyolefin containers are stable for at least 25 hours at ambient temperatures (20-23°C). (Waugh, et al. (1990) Am. J. Hosp. Pharm. 48, 1520). Although these concentrations have exhibited stability for the above periods of time, they are not meant to limit the practice of the present invention wherein any concentration of paclitaxel may be utilized.

[0025] All solutions of paclitaxel exhibit a slight haziness directly proportional to the concentrations of drug and time elapsed after preparation. Formulation of a small number of fibers in the solution (within acceptable limits established by the USP Particulate Matter Test for LVP's) has been observed after preparation of paclitaxel infusion solutions. While particulate formation does not indicate loss of drug potency, solutions exhibiting excessive particulate matter formation should not be used. Therefore, when administering via continuous infusion, in-line filtration may be necessary and can be accomplished by incorporating a hydrophilic, microporous filter with a pore size no greater than 0.22 microns (IVEX-HP In Line Filter Set-SL, 15", Abbott model # 4525 or equivalent) into the fluid pathway distal to an infusion pump.

[0026] Paclitaxel must be prepared in non-plasticized solution containers (e.g., glass, polyolefin, or polypropylene) due to leaching of diethylhexylphthlalate (DEEP) plasticizer from polyvinyl chloride (PVC) bags and intravenous tubing. Paclitaxel must not be administered through PVC intravenous or injection sets. Therefore, polyolefin- or polyethylene-line sets, such as IV nitroglycerin sets (or equivalent) should be used to connect the bottle or bag (containing the paclitaxel solution for a continuous infusion) to the IV pump, a 0.22 micron filter is then attached to the IV set, and then may be directly attached to the patient's central access device If necessary, a polyolefin-line extension set (Polyfin™ Extension Set, MiniMed Technologies, Model #126) can be used to provide additional distance between the IV pump and the patient's central access device.

[0027] A human dosing schedule can consist of (but not be limited to) 24-hour continuous IV pretreatment with up to about 0.5-2 mg/kg (20-80 mg/m^2) prior to the vascular procedure, about 0.25-2 mg/kg (10-80 mg/m^2) continuous IV infusion over the 24 hours post-procedure, then about 0.25- 2 mg/kg (10-80 mg/m^2) continuous IV Infusion over 24 hours every 21 days for 1 to 6 cycles. Such a dosage is significantly lower than that used to treat human cancers (approximately 4-6 mg/kg).

[0028] One category of paclitaxel use would encompass the primary prevention, or the attenuation, of vascular disease (atherosclerosis) development. Certain of these applications (examples of which include the prevention of cardiac allograft (transplant) atherosclerosis, the multi-organ system failure resulting from the vascular complications of diabetes mellitus or accelerated, medically-refractory atherosclerosis in patients who are poor surgical candidates) may require the later treatment cycles to be continuous low-dose (1-5 mg/m^2/day) IV infusions over 5-7 days. Each of the paclitaxel treatments will generally require pretreatment with dexamethasone 20 mg orally 14 and 7 hours prior to paclitaxel, diphenhydramine 50 mg IV and cimetidine 300 mg IV 30 min prior to paclitaxel to minimize potential episodes of allergic reaction. Additional applications that may not be associated with a surgical procedure include treatment of vascular fibromuscular dysplasia, polyarteritis nodosa, and Takayasu's arteritis. Each of the aforementioned applications may also be amenable to selective, localized application of sustained-release preparations of paclitaxel, paclitaxel derivatives or deuterium oxide which would enable high dosage local drug delivery with little systemic toxicity.

[0029] Additionally, water soluble derivatives of paclitaxel can also be used in the present invention. The water soluble derivatives of paclitaxel, as described in U.S. Pat. No. 5,157,049 to Haugwitz, et al. (incorporated herein by reference) include, but are not limited to, 2'-succinyl-paclitaxel; 2'-succinyl-paclitaxel triethanolamine; 2'-glutaryl-paclitaxel; 2'-glutaryl-paclitaxel triethanolamine salt; 2'-O-ester with N-(dimethylaminoethyl) glutamide; 2'-O-ester with N-(dimethylaminoethyl) glutamide hydrochloride salt. These water soluble paclitaxel derivatives can be administered in a dosage schedule analogous to that given above for paclitaxel with the appropriate modifications pending clarification of the pharmacokinetics of these agents.

[0030] A pharmaceutical composition comprising an effective amount of water soluble derivative of paclitaxel as an active ingredient is easily prepared by standard procedures well known in the art, with pharmaceutically acceptable non-toxic sterile carriers, if necessary. Such preparations could be administered orally or in injectable form, or directly to an affected area, to a patient at risk of developing or suffering from atherosclerosis to prevent or reduce the development of the disease.

[0031] The following examples illustrate the effectiveness of paclitaxel, paclitaxel derivatives and deuterium oxide in inhibiting the proliferation and migration of vascular smooth muscle cells.

Example 1

[0032] The in vitro ability of cultured VSMCs, pretreated with different paclitaxel concentrations, to invade filters coated with reconstituted basement membrane proteins was tested to evaluate how paclitaxel-induced microtubule

bundling would impair cell processes necessary for in vivo neointimal formation.

[0033] Vascular Smooth Muscle Cells (VSMCs) were isolated by collagenase/elastase enzymatic digestion of the medial layers of the rat aorta obtained from 6 month old Wistar rats. The cells were maintained in culture with 10% fetal calf serum, high glucose DMEM, and amino acid supplement. Cell cultures were maintained at 37°C in 5% CO_2.

[0034] After 18-hour paclitaxel pre-treatment in culture, cells were fixed in 3.7% formalin, permeabilized with 1 % Triton X-100, and polymerized tubulin was labelled with mouse anti-β-tubulin antibody (SMI 62 monoclonal antibody to polymerized β-tubulin, Paragon Biotec, Inc., Baltimore, MD). Secondary labelling was achieved with silver-enhanced, 1 nm gold-conjugated rabbit anti-mouse antibody (Goldmark Biologicals, Phillipsburg, NJ). Representative light photomicrographs from (A) control, (B) 0.1 nM paclitaxel, (C) 1 nM paclitaxel, and (D) 10 nM paclitaxel treated VSMCs are shown in Figure 4.

[0035] Chemoinvasion (Boyden chamber) assays were performed using modified Boyden chamber (Albini, et al. (1987) Cancer Res., 47:3239-3245), comprising an upper chamber separated from a lower chamber by a porous PVPF filter. PVPF filters (8 µm pore diameter, Nucleopore Filters, Pleasonton, CA) were coated and air dried consecutively with solutions containing 100 µg/ml type I collagen, 5 µg/ml fibronectin, and 5 µg reconstituted basement membrane (produced from the Englebreth-Holm-Swarm tumor (Kleinman, et al. (1986) Biochemistry, 25:312-318), producing a continuous 10 µm thick coating of matrix material. Boyden chambers were assembled by adding 10 ng/ml PDGF BB in DMEM to the lower (chemoattractant) chamber. Cells (approximately 200,000) suspended in DMEM containing 0.1% BSA were then added to the upper chamber. Some of the cells used in this assays were pretreated 18 hours with paclitaxel (concentration 30 pM to 100 nM) in culture. In the paclitaxel-treated groups, paclitaxel was added to the upper and lower chambers at the same concentration as that used for pretreatment. The chambers were then incubated for 4 hours at 37°C in a 5% CO_2 atmosphere. At the end of the incubation period, the cells were fixed and stained with hematoxylin and eosin. The cells on the upper surface (non-invaders) were mechanically removed and the cells on the underside of the filter (invaders) were counted under 400 x magnification (four random fields were counted per filter and all experiments were run in triplicate, and each triplicate assay was repeated at least three times on separate occasions using different VSMC preparations). Chemotaxis was assayed in analogous fashion in the Boyden chambers described above, except that the reconstituted basement membrane was omitted. This chemoinvasion assay is accepted by those skilled in the art as exhibiting high correlation between invasiveness in vitro and cellular behavior as it occurs in vivo (Iwamoto, Y., et al. (1992) Advances In Experimental Medicine & Biology, 324:141-9).

[0036] Using the PDGF-BB as an attractant, paclitaxel inhibited VSMC invasion with half-maximal inhibitory concentration of 0.5 nM. Paclitaxel caused essentially complete inhibition at 100 nM, and significant inhibition was still resolvable at 30 pM (the lowest dose used) (Figure 1). A chemotaxis assay (filter coated only with fibronectin and collagen I, without basement membrane proteins occluding the filter pores) with PDGF-BB as the attractant was performed in analogous fashion, yielding the identical outcome. These results demonstrate that paclitaxel, at least at nanomolar drug levels, inhibits VSMC invasion primarily via inhibition of locomotion and/or shape changes, rather than by inhibiting cellular secretion of collagenases and metalloproteinases, which are known to be necessary for VSMC to penetrate basement membrane proteins in this assay.

[0037] Gelatinase zymography was performed on the supernatants removed after the 4 hour conclusion of the Boyden assays described above. Gelatin-degrading proteinases secreted into the media by VSMCs were analyzed by non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 10% polyacrylamide gels containing 0.1% (w/v) gelatin. Following electrophoresis, the gelatinases were renatured by incubating the gel for 30 min. at 23°C in 2.5% (v/v) Triton X-100 followed by 18 hour incubation at 37°C in 0.2 M NaCl, 5 mM $CaCl_2$ 0.02% Brij 35 (w/v), 50 mM Tris-HCl (pH 7.6). The gels were stained for 90 minutes with 0.5% Coomassie Brilliant Blue G-250 and destained with 10% acetic acid, 40% methanol. Gelatinolytic activity was indicated by a clear band against the background of blue-stained gelatin.

[0038] These gelatinase zymography assays from the Boyden chamber invasion experiments confirm that the level of VSMC collagenase secretion did not vary significantly over the paclitaxel range 30 pM to 100 nM, compared to control (Figure 2, inset).

Example 2

[0039] To confirm the fact that microtubule stabilization and hyperpolymerization is the critical and sufficient factor involved in paclitaxel-inhibition of VSMC invasiveness, the chemoinvasion (Boyden chamber) assay was run with deuterium oxide (^2H_2O, heavy water). Deuterium oxide enhances microtubule/tubulin polymerization via a mechanism distinct from that of paclitaxel. A combination of the isotope and solvent effects of deuterium oxide reversibly increases microtubule polymerization both by reducing the critical concentration for polymerization for αβ-tubulin heterodimers via enhanced tubulin hydrophobic interactions (Itoh, T.J., et al. (1984) Biochim. Biophys. Acta., 800:21-27), and by converting a population of unpolymerizable tubulin to the polymerizable form (Takahashi, T.C., et al. (1984) Cell Struct. Funct., 9:45-52).

[0040] VSMC's were isolated by collagense/elastase enzymatic digestion of the medial layers of the rat aorta obtained from 6 month old Wistar rats. The cells were maintained in culture with 10 % fetal calf serum, high glucose DMEM, and amino acid supplement. Cell cultures were maintained at 37°C in 5% CO_2.

[0041] In deuterium oxide-treated cells, ^2H_2O (v/v) was substituted for water (H_2O) in the preparation of the DMEM from concentrated stock. After 18-hour deuterium oxide pre-treatment in culture, cells were fixed in 3.7% formalin, permeabilized with 1 % Triton X-100, and polymerized tubulin was labelled with mouse anti-β-tubulin antibody (SMI 62 monoclonal antibody to polymerized β-tubulin, Paragon Biotec, Inc., Baltimore, MD). Secondary labelling was achieved with silver-enhanced, 1 nm gold-conjugated rabbit anti-mouse antibody (Goldmark Biologicals, Phillipsburg, NJ). Representative light photomicrographs from (5A) control, and (5B) 75% deuterium oxide treated VSMCs are shown in Figures 5A-5B.

[0042] Chemoinvasion assays were performed using a modified Boyden chamber, consisting of an upper chamber separated from a lower chamber by a porous PVPF filter. PVPF filters (8 μm pore diameter, Nucleopore Filters, Pleasonton, CA) were coated and air dried consecutively with solutions containing 100 μg/ml type I collagen, 5 μg/ml fibronectin, and 5 μg reconstituted basement membrane (produced from the Englebreth-Holm-Swarm tumor), producing a continuous 10 μm thick coating of matrix material. Boyden chambers were assembled with PDGF-BB 10 ng/ml in DMEM in the lower (chemoattractant) chamber, then cells (approximately 200,000) suspended in DMEM containing 0.1% BSA were added to the upper chamber. Some of the cells used in these assays were pretreated 18 hours with deuterium oxide (25%, 50%, or 75% v/v substitution for H_2O) in culture. In the deuterium oxide-treated groups, ^2H_2O substituted DMEM (v/v) was added to the upper and lower chambers at the same concentration as that used for pretreatment. The chambers were then incubated for 4 hours at 37°C in a humidified 5% CO_2 atmosphere. At the conclusion of the experiment, the filters were removed and the cells were fixed and stained with hematoxylin and eosin. After the cells on the upper surface of the filter (non-invaders) were mechanically removed, the cells on the underside (invaders) were counted under 400 X magnification (four random fields were counted per filter and all experiments were run in triplicate).

[0043] Pretreating cultured VSMCs for 18 hours with 25%, 50% or 75% deuterium oxide caused dose-dependent microtubule hyperpolymerization similar to that observed with paclitaxel. This treatment likewise inhibited PDGF-mediated VSMC Boyden chamber invasion in a dose-dependent fashion, achieving half-maximal inhibition at 25% ^2H_2O, and nearly complete inhibition at 75% ^2H_2O (Figure 3).

Example 3

[0044] In addition to cell recruitment and migration, the various growth regulatory molecules elaborated after arterial injury, such as PDGF and bFGF, are also implicated in mitogenesis and cellular proliferation. To measure the effect of paclitaxel on VSMC DNA synthesis, [^{3}H]thymidine incorporation was measured. VSMCs were plated at 4.5 x 10^4 on 24-well plates. Following 5 hr. incubation in 10% FCS+DMEM, 0.5 mCi [^{3}H] thymidine was added and the incubation continued for an additional 16 hrs. Cells were washed twice with phosphate-buffered saline, extracted with 10% TCA for 2 hrs. on ice, then centrifuged at 2,000 g for 10 mins. Supernatants were decanted and pellets were solubilized in 0.5 ml of 1 N NaOH. After neutralizing with 0.5 ml of 1 N HCl, [^{3}H] thymidine uptake was determined by a Beckman liquid scintillation counter. VSMCs were treated with the various concentrations of paclitaxel for both the 18 hr. prior to the addition of the thymidine and during thymidine incorporation. Each condition of these experiments was performed in triplicate.

[0045] Paclitaxel inhibited cultured VSMC [^{3}H] thymidine incorporation, an index of cell division, in a dose-dependent fashion, with a half-maximal inhibitory concentration of 5 nM. Paclitaxel caused essentially complete inhibition at 100 nM, and significant inhibition was resolvable at 1 nM (Figure 1). That this inhibitory profile differs somewhat from that of invasion and chemotaxis, demonstrating one log-concentration-unit lower sensitivity but with steeper concentration-dependence, likely arises because of the considerably different roles played by mircotubules between these processes. Paclitaxel also inhibited PDGF-BB-stimulated c-fos mRHA expression in this cultured VSMC model, in a dose-dependent fashion, with a half-maximal inhibitory concentration of 1 nM, with essentially complete inhibition above 20 nM. Thus, inhibition of immediate early gene induction is another important mechanism by which paclitaxel blocks growth factor stimulation in VSMCs, and may underlie, at least in part, the thymidine incorporation results.

[0046] Thus, paclitaxel significantly inhibits cultured VSMC in vitro invasion and proliferation through interference with microtubule function, disrupting locomotion and the ability to alter shape, as well as growth-factor stimulated early gene expression and cell proliferation, at concentrations one hundred - to one thousand-fold lower than used to treat human cancer.

Example 4

[0047] Incorporation of the thymidine analog, bromodeoxyuridine (BrDU) was measured to determine the effect of

deuterium oxide on VSMC DNA synthesis. VSMCs were plated at 4.5 x 10^4 on 24-well plates. Following 20 hr incubation in 10% FCS+DMEM at various ^2H_2O concentrations, 10 μM BrDU was added and the incubation continued for an additional 4 hr. Cells were washed twice with phosphate-buffered saline (PBS) and fixed with 100% methanol (-20°C) for 10 minutes. The cells were incubated for 2 hr with 1N HCl to denature the DNA, and subsequently washed 4 times in PBS. Mouse monoclonal BrDU antibody (Boehringer Mannheim) in 2% BSA-PBS was incubated with cells for 1 hr. After PBS wash, goat anti-mouse antibody conjugated with alkaline phosphatase was added. Cell nuclei containing BrDU substituted for thymidine stained red with alkaline phosphatase substrate, while all other nuclei stained blue. The fraction of BrDU-positive nuclei was compared between control (defined as 100%) and that of the deuterium oxide-pretreated groups.

[0048] The results indicated that deuterium oxide, similar to paclitaxel, inhibited cultured VSMC proliferation and DNA synthesis in a dose-dependent fashion, consistent with the critical balance of microtubule-tubulin dynamics in VSMC proliferation.

[0049] While paclitaxel and deuterium oxide potentially have multiple intracellular effects, the coincidence of their parallel effects on microtubules (despite different mechanisms of action) and on VSMC functionality at multiple levels, indicates that the common microtubule stabilizing mechanism of action is responsible for the observed functional changes. Thus based on the results of experiments with both paclitaxel and deuterium oxide, it is evident that microtubules are involved in the control of the most critical and sensitive intracellular mechanisms necessary for VSMCs to undergo the multiple transformations involved in the development of atherosclerosis after arterial injury, making microtubules particularly strategic targets to influence the outcome.

Example 5

[0050] Under a protocol approved by the National Institute on Aging Animal Care and use Committee, 6 month Wistar rats from the GRC colony were anesthetized with 20 mg/kg body weight pentobarbital, 2 mg/kg body weight ketamine, and 4 mg/kg body weight xylazine intraperitoneally. The left external carotid artery was cannulated with 2-French Fogarty embolectomy catheter, inflated with saline and passed three times up and down the common carotid artery to produce a distending, deendothelializing injury. The animals were treated with 2 mg/kg body weight paclitaxel solution or the control animals with vehicle alone (13.4 ml/kg body weight per day of 1:2:2:165 DMSO:Cremophor EL:Dehydrated ethanol:phosphate buffered saline) by intraperitoneal injection beginning 2 hours after injury. The paclitaxel solution or vehicle alone was administered once daily, as an intraperitoneal injection, for the next 4 days. After 11 days the animals (8 paclitaxel-treated and 10 vehicle-treated) were anesthetized as above and the carotid artery was isolated and fixed in 10% buffered formalin and embedded in paraffin. Cross sections of the carotids were mounted on microscope slides and stained with hematoxylin and eosin stain. The image of the carotid artery was projected onto a digitizing board and the cross sectional areas of the intima and the media were measured. The results are shown in Figure 2.

[0051] Quantitative analysis of injured carotid segments showed that paclitaxel treatment reduced the neointimal area by 70% compared to vehicle treated animals (Table I) (*P < 0.001; †P=NS; ‡P < 0.001). Several of the paclitaxel-treated animals showed virtually no discernable neointima (in the presence of denuded endothelium, proving injury), while all vehicle treated animals demonstrated at least modest neointimal thickening.

[0052] While the in vivo systemic paclitaxel dose used in these experiments (2 mg/kg) is significantly lower than that ordinarily used to treat human cancers (approximately 3-6 mg/kg), dramatically lower systemic dosing with sustained or even improved efficacy could be possible combining a pretreatment regimen with the optimal treatment duration. Furthermore, since the goal of therapy is to keep the "activated" VSMCs in check, or preferably to prevent activation in the first place, until the stimulus for growth and migration has resolved (rather than causing cytotoxicity resulting in cell death), the goal of short-term therapy with limited toxicity may be possible in humans. Ultimately, local sustained-release delivery systems may offer the best solution to prevent atherosclerosis, enabling high local concentrations of drug delivery and essentially eliminating problems of systemic toxicity. Drug delivery systems that can be valuable include drug-impregnated polymer-coated metallic stents, biodegradable drug-eluting polymer stents, and genetically primed endothelial cells to coat metallic stents or be delivered directly as a local endothelial cell covering. (Muller, D. W.M. et al. (1991) JACC 17:126b-131b). These systems allow safe use of a chemotherapeutic agent without systemic side effects. Alternatively, treatment may involve a period of pretreatment (i.e., before vascular surgery) via continuous intravenous infusion for a period of time, followed by a different therapy during (local, direct delivery) or after (oral, injection) surgery.

[0053] The above examples teach paclitaxel's, paclitaxel derivative's and deuterium oxide's potential beneficial uses to prevent artery blockage and thereby reduce the possibility of, or prevent, heart attacks, strokes, kidney failure and renal dialysis, blindness, limb amputations, nerve loss, need for corrective vascular surgery/angioplasty or organ transplantation, and premature and permanent disability requiring chronic hospitalization. The invention has been described in detail, but it will be understood that the invention is capable of other different embodiments.

TABLE 1

Group	Intima (mm^2)	Media (mm^2)	I/M
Vehicle	0.09±0.01	0.14±0.01	0.66±.08
Paclitaxel	0.03±0.01*	0.16±0.02†	0.18±.04‡

Claims

1. A drug delivery system comprising a means for locally delivering paclitaxel, a paclitaxel derivative or deuterium oxide other than by intravenous injection or infusion in a therapeutically effective amount to reduce or prevent the development of atherosclerosis.

2. A drug delivery system according to claim 1, wherein the means for locally delivering paclitaxel, a paclitaxel derivative or deuterium oxide comprise polymer-coated metallic stents and polymer stents.

3. A drug delivery system according to claim 2, wherein the stents comprise biodegradable drug-eluting polymer stents.

4. Use of deuterium oxide in the manufacture of a pharmaceutical composition for the prevention of a fibroproliferative vascular disease in a patient.

5. Use of paclitaxel or a paclitaxel derivative in the manufacture of a pharmaceutical composition for the prevention or reduction of atherosclerosis in a patient.

6. The use of claim 5, wherein said paclitaxel derivative is a water-soluble paclitaxel derivative selected from the group consisting of a 2'-succinyl-paclitaxel; 2'-succinyl-paclitaxel triethanolamine; 2'glutaryl-paclitaxel; 2'-glutaryl-paclitaxel triethanolamine salt; 2'-O-ester with N-(dimethylaminoethyl) glutamide; and 2'-O-ester with N-(dimethylaminoethyl) glutamide hydrochloride salt.

7. The use of any of claims 4 to 6, wherein said treatment comprises the systemic delivery of said pharmaceutical preparation.

8. The use of any one of claims 4 to 7, wherein said treatment comprises the local delivery of said pharmaceutical preparation.

9. The use of claim 8, wherein the local delivery comprises a local sustained release delivery system.

10. The drug delivery system according to claim 2, wherein the paclitaxel derivative comprises a water-soluble paclitaxel derivative selected from 2'-succinyl-paclitaxel; 2'-succinyl-paclitaxel triethanolamine; 2'-glutaryl-paclitaxel; 2'-glutaryl-paclitaxel triethanolamine salt; 2'-O-ester with N-(dimethylaminoethyl)glutamide; and 2'-O-ester with N-(dimethylaminoethyl) glutamide hydrochloride salt.

Patentansprüche

1. System zur Verabreichung eines Arzneistoffs, umfassend ein Mittel zur lokalen, von intravenöser Injektion oder Infusion verschiedenen Verabreichung von Paclitaxel, einem Paclitaxel-Derivat oder Deuteriumoxid in einer therapeutisch wirksamen Menge, um die Entwicklung von Atherosklerose zu verlangsamen oder zu verhindern.

2. System zur Verabreichung eines Arzneistoffs gemäß Anspruch 1, wobei das Mittel zur lokalen Verabreichung von Paclitaxel, einem Paclitaxel-Derivat oder Deuteriumoxid polymerbeschichtete Metall- oder Polymer-Stents umfasst.

3. System zur Verabreichung eines Arzneistoffs gemäß Anspruch 2, wobei die Stents biologisch abbaubare, Arzneistoffe eluierende Polymer-Stents umfassen.

4. Verwendung eines Deuteriumoxids bei der Herstellung eines Arzneimittels zur Verhinderung einer fibroproliferativen Gefäßerkrankung bei einem Patienten.

5. Verwendung von Paclitaxel oder einem Paclitaxel-Derivat bei der Herstellung eines Arzneimittels zur Verhinderung oder Verringerung von Atherosklerose bei einem Patienten.

6. Verwendung gemäß Anspruch 5, wobei das Paclitaxel-Derivat ein wasserlösliches Paclitaxel-Derivat ist, ausgewählt aus 2'-Succinylpaclitaxel, 2'-Succinylpaclitaxeltriethanolamin, 2'-Glutarylpaclitaxel, 2'-Glutarylpaclitaxeltriethanolaminsalz, 2'-O-Ester mit N-(Dimethylaminoethyl)glutamid und 2'-O-Ester mit N-(Dimethylaminoethyl)glutamidhydrochloridsalz.

7. Verwendung gemäß einem der Ansprüche 4 bis 6, wobei die Behandlung die systemische Verabreichung des Arzneimittels umfasst.

8. Verwendung gemäß einem der Ansprüche 4 bis 7, wobei die Behandlung die lokale Verabreichung des Arzneimittels umfasst.

9. Verwendung gemäß Anspruch 8, wobei die lokale Verabreichung ein lokales Verabreichungssystem mit kontinuierlicher Freisetzung umfasst.

10. System zur Verabreichung eines Arzneistoffs gemäß Anspruch 2, wobei das Paclitaxel-Derivat ein wasserlösliches Paclitaxel-Derivat umfasst, ausgewählt aus 2'-Succinylpaclitaxel, 2'-Succinylpaclitaxeltriethanolamin, 2'-Glutarylpaclitaxel, 2'-Glutarylpaclitaxeltriethanolaminsalz, 2'-O-Ester mit N-(Dimethylaminoethyl)glutamid und 2'-O-Ester mit N-(Dimethylaminoethyl)glutamidhydrochloridsalz.

Revendications

1. Système d'administration de médicaments comprenant un moyen pour délivrer localement le paclitaxel, un dérivé du paclitaxel ou l'oxyde de deutérium, autre que par injection ou perfusion intraveineuse, en quantité thérapeutiquement efficace pour réduire ou prévenir le développement d'une athérosclérose.

2. Système selon la revendication 1, dans lequel le moyen pour délivrer localement le paclitaxel, un dérivé du paclitaxel ou l'oxyde de deutérium comprend des extenseurs métalliques recouverts de polymère et des extenseurs polymériques.

3. Système selon la revendication 2, dans lequel les extenseurs comprennent des extenseurs polymériques biodégradables éluant le médicament.

4. Utilisation de l'oxyde de deutérium dans la fabrication d'une composition pharmaceutique pour la prévention d'une maladie vasculaire fibroproliférative chez un patient.

5. Utilisation du paclitaxel ou d'un dérivé du paclitaxel dans la fabrication d'une composition pharmaceutique pour la prévention ou la réduction de l'athérosclérose chez un patient.

6. Utilisation selon la revendication 5, dans laquelle ledit dérivé du paclitaxel est un dérivé du paclitaxel hydrosoluble sélectionné parmi le groupe de composés suivants : 2'-succinyl-paclitaxel ; 2'-succinyl-paclitaxel triéthanolamine ; 2'-glutaryl-paclitaxel ; 2'-glutaryl-paclitaxel, sel de triéthanolamine ; 2'-O-ester avec le N-(diméthylaminoéthyl) glutamide ; et 2'-O-ester avec le chlorhydrate de N-(diméthylaminoéthyl) glutamide.

7. Utilisation selon l'une quelconque des revendications 4 à 6, dans laquelle ledit traitement comprend l'administration systémique de ladite préparation pharmaceutique.

8. Utilisation selon l'une quelconque des revendications 4 à 7, dans laquelle ledit traitement comprend l'administration locale de ladite préparation pharmaceutique.

9. Utilisation selon la revendication 8, dans laquelle l'administration locale se fait par un système d'administration locale à libération prolongée.

10. Système d'administration de médicaments selon la revendication 2, dans lequel le dérivé du paclitaxel comprend un dérivé du paclitaxel hydrosoluble sélectionné parmi les composés suivants : 2'-succinyl-paclitaxel ; 2'-succinyl-paclitaxel triéthanolamine ; 2'-glutaryl-paclitaxel ; 2'-glutaryl-paclitaxel, sel de triéthanolamine ; 2'-O-ester avec le N-(diméthylaminoéthyl) glutamide ; et 2'-O-ester avec le chlorhydrate de N-(diméthylaminoéthyl) glutamide.

FIG. 1

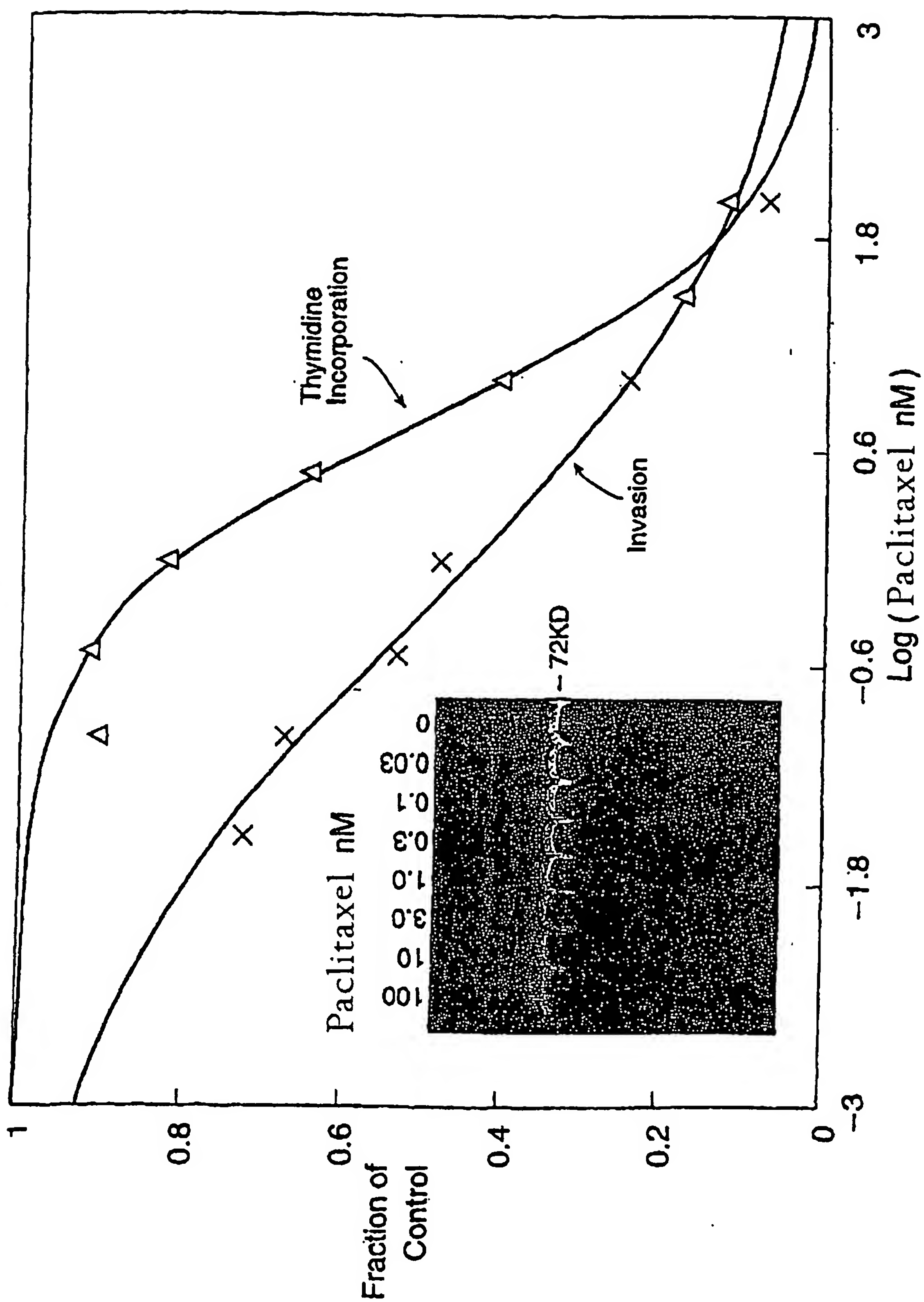
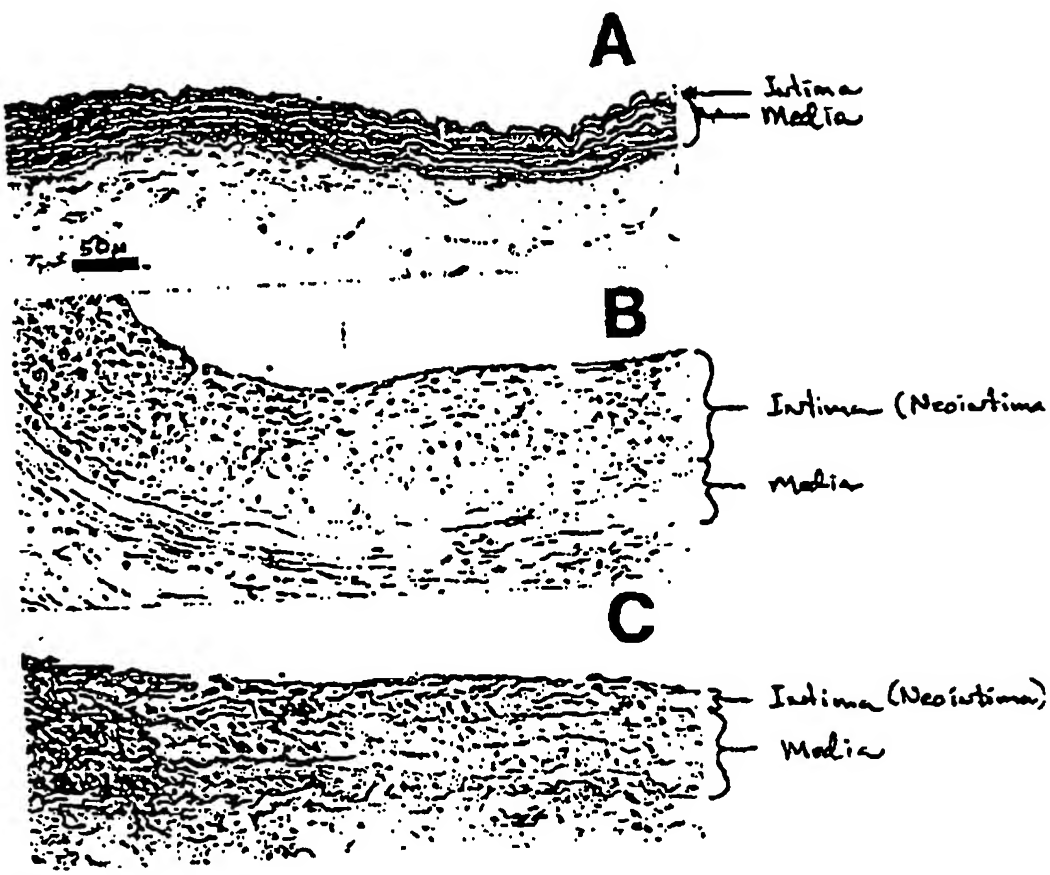

Fraction of Control
1
0.8
0.6
0.4
0.2
0
−3
−1.8
−0.6
0.6
1.8
3
Log (Paclitaxel nM)
Thymidine Incorporation
Invasion
Paclitaxel nM
100
10
3.0
1.0
0.3
0.1
0.03
0
72KD

Figure 2



TAXOL INHIBITS THE ACCUMULATION OF INTIMAL SMOOTH MUSCLE CELLS 11 DAYS AFTER BALLOON CATHETER INJURY OF RAT CAROTID ARTERY. HEMATOXYLIN- AND EOSIN-STAINED CROSS SECTIONS OF BALLOON-CATHETERIZED RAT COMMON CAROTID ARTERIES 11 DAYS POST-PROCEDURE.
(A) UNINJURED ; (B) INJURED + VEHICLE ; (C) INJURED + TAXOL

Figure 3

VSMC Invasion and BrDU Incorportion:
Effect of Deuterium Oxide

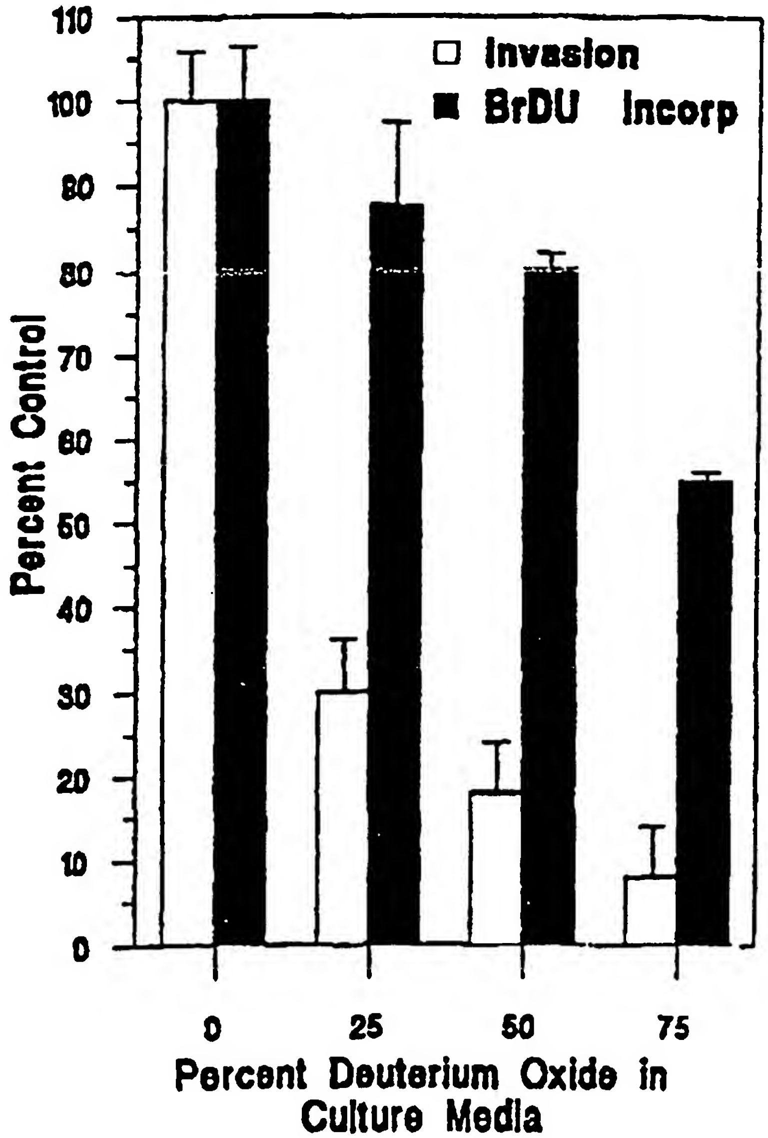


Figure 4

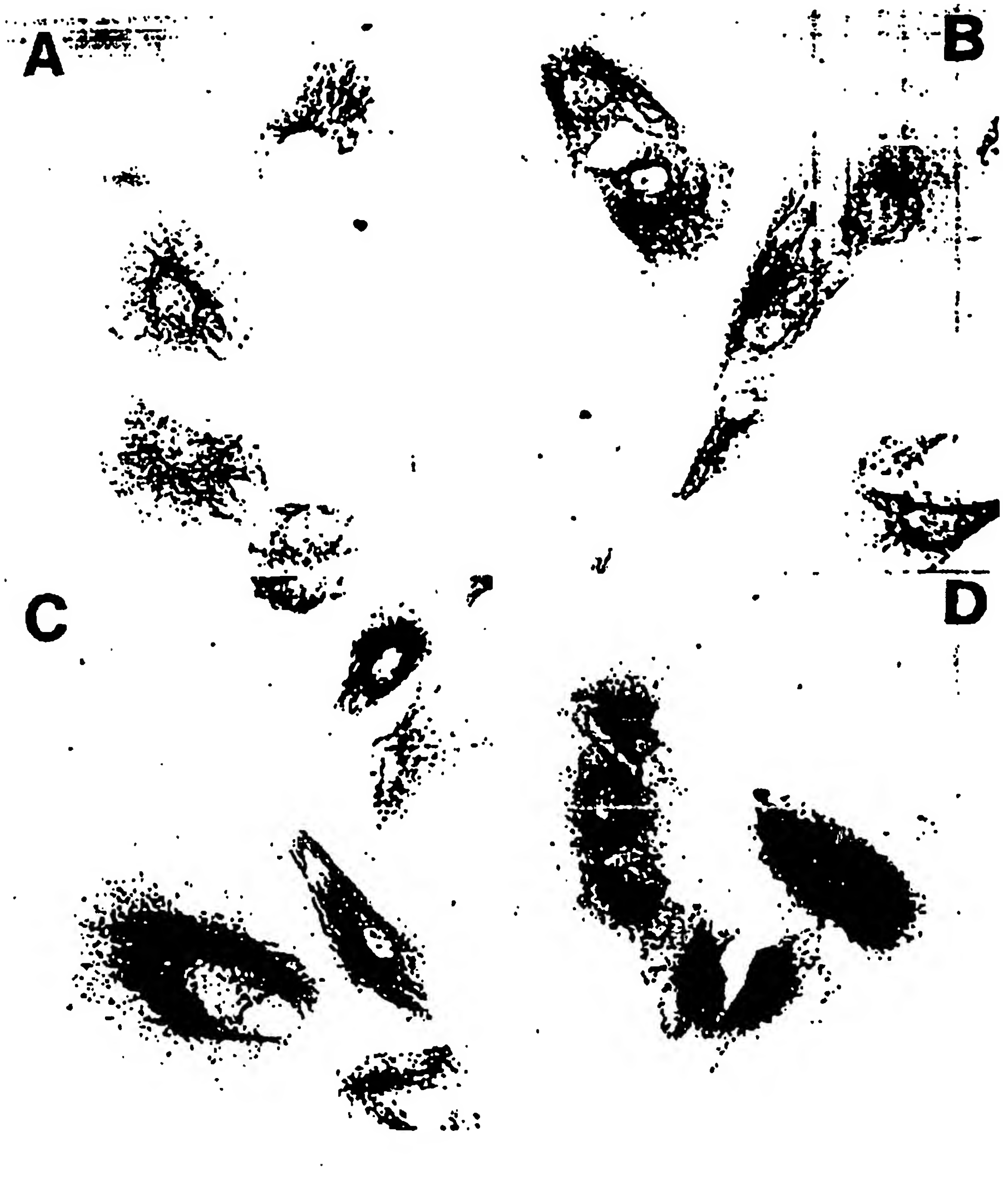
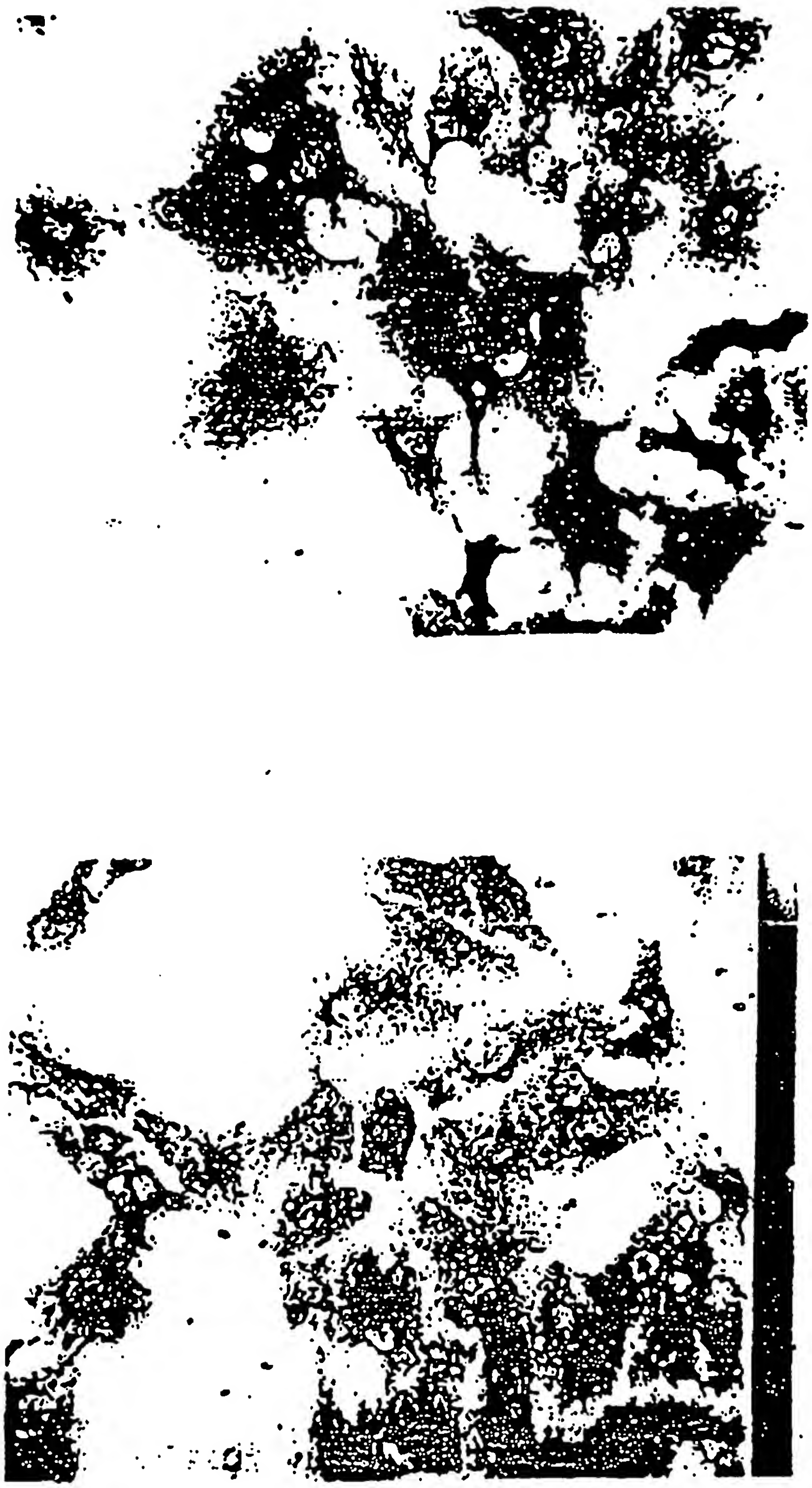


Figure 5.



control VSMC non serum starved stimulation

A. CONTROL

75% D2O VSMC non-serum starved stimulation

B. 75% DEUTERIUM OXIDE